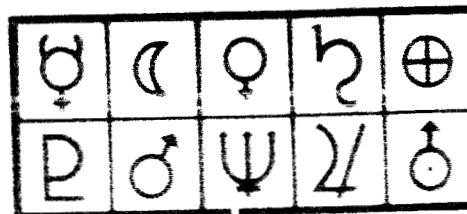


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PLANETARY QUARANTINE

EVALUATION OF THE EFFICIENCY OF A CLASS 100 LAMINAR-FLOW CLEAN ROOM FOR VIABLE CONTAMINATION CLEANUP

(Part I of Microbiological Studies Relating
to Clean Environments)

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ABSTRACT

A laminar-flow wall-to-floor clean room was challenged with Bacillus subtilis spores and then tested for efficiency of cleanup using both electronic and viable particle detection systems. The results confirmed the extreme efficiency of laminar-flow systems in reducing airborne viable particles to an absolute minimum.

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EVALUATION OF THE EFFICIENCY OF A CLASS 100 LAMINAR-FLOW CLEAN ROOM FOR VIABLE CONTAMINATION CLEANUP

Introduction

Previous studies have shown that the laminar-flow principle is highly efficient in removing non-viable particulate contamination from clean room systems. Indeed, the efficiency of these systems may be challenged with dioctyl phthalate smoke, and rapid clearance of the system may be observed, photographed, and monitored by various electronic particle counters.¹

For many applications of clean rooms it is desirable also to achieve an environment free of viable particles, so studies were initiated to evaluate the efficiency of these systems in reducing or eliminating bacterial and fungal contamination. Initial studies² indicated that the laminar-flow clean room was indeed very free from viable contamination when compared with contemporary techniques and standards of cleanliness. A confirmation study³ on the presence of viable particles in such a clean room indicated not only that the room was remarkably free of indigenous contamination but that, when challenged with concentrated aerosols of Serratia marcescens, the room attained absolute cleanup within 1 minute. Serratia marcescens, however, does not form spores and it is certain that many of the bacteria were by nature no longer viable at the close of the sampling period. In addition, in order that electronic particle monitors such as the Royco PC 200A particle counter can be used, cultures must be washed and suspended in distilled water, since even an 0.85 percent saline will cause droplet nuclei to register as particles. Consequently, the preliminary evaluations^{2,3} have been extended, utilizing Bacillus subtilis spores to avoid the pitfalls associated with the viability of bacterial suspensions when treated in the necessary manner.

The present investigation was made to determine the efficiency of laminar-flow devices in reducing viable particulate contamination.

Materials and Methods

Bacillus subtilis var. niger (B. globigii) was seeded heavily onto the surface of Bacto-TAM sporulation agar and incubated at 42°C for 72 hours. The growth was suspended in normal saline, and heat shocked at 80°C for 10 minutes. This spore suspension was then used to inoculate very heavily the surface of TAM sporulation agar plates; the plates were incubated at 42°C for 48 hours, after which spore stains showed the culture to consist of more than 98 percent spores. A bent stainless steel rod was used to harvest the growth from the surface, and the growth was then suspended in sterile distilled water. The suspension (cooled in an ice bath) was sonicated for 45 minutes with a Branson Sonifier.

Following sonication, spore stains revealed almost total spores along with some cellular debris. The suspension was centrifuged in the cold at 12,000 x g for 10 minutes, then the supernatant was discarded and the sediment spores were re-suspended in sterile distilled water and centrifuged at 2,000 x g for 5 minutes. The spores were re-suspended in sterile distilled water, thoroughly shaken, aliquoted in 2-ml quantities in sterile serum bottles, frozen at -20°C, and stored at -20°C until used. This stock slurry of spores gave an approximate plate count of 4.7×10^9 per ml throughout the test period.

The original Whitfield clean room⁴ was used for all experiments. This room is a laminar-flow type of clean room with wall-to-floor airflow. All operations were accomplished, from a remote location outside the room, through large-diameter (3/4"-1" tygon tubing. All manipulations were performed in a clean environment made possible by positioning a curtained laminar-downflow unit immediately adjacent to the sampling ports.

The appropriate dilution (in distilled water) of spore suspension was sonicated for 30 seconds immediately prior to use, and then aerosolized for 1 minute in a De Vilbiss No. 40 nebulizer, with the room circulation turned off. Nitrogen at 5 pounds pressure delivered a volume of approximately 0.5 ml of aerosol through a tube to a mixing fan inside the room. The fan ran for 5 minutes following aerosolization so that the aerosol concentrations in the room might become stabilized.

Two Andersen samplers functioning at 1 cfm were used for each experiment; their flow rate was monitored continuously. The first Andersen sampler was used to sample the aerosol concentration in the room while the air circulation was turned off, immediately following the stabilization of the aerosol. The second sampler was used to sample "clean" air after the room circulation system had run for 1 minute. Each sampler had separate tubing, and precautions were taken to avoid sampling a surface residual of spores because of contamination of the sample tubes. The sampling protocol was as follows:

1. Room circulation turned off.
2. Spore suspension aerosolized for 1 minute by a De Vilbiss No. 40 nebulizer.
3. Mixing fan operated for 5 minutes.
4. Mixing fan stopped; first Andersen sampler functioned for 1 minute.
5. Room circulation turned on; protective coverings removed from clean sampler tubes.
6. After 1 minute of room circulation, second Andersen sampler functioned for 5 minutes.

All Andersen samplers were sterilized at 121°C for 15 minutes in an autoclave. Trypticase soy agar was used as a sampling medium. All plates were prepared in a laminar-downflow bench, incubated for sterility at 28°C for 48 to 72 hours, and stored at 4°C until used. All Andersen samplers were loaded and unloaded in a laminar-downflow clean bench. All plates were incubated at 28°C for 48 to 72 hours and counted.

A Royco PC 200A particle counter, modified to give a rapid cleanup, was run continuously throughout each experiment. It monitored the total number of particles 0.3 μ and larger at 1-minute intervals.

Following aerosolization, the slurry and dilutions used were refrigerated until plate counts could be made. Plate counts were also incubated at 28°C for 48 to 72 hours prior to counting.

Results and Discussion

The results of the aerosolization of Bacillus subtilis spores into a room and subsequent clearance of the room by laminar-flow air circulation are shown in Tables I and II.

Table I shows the results as measured by the Royco PC 200A,* which counted all particles 0.3 μ and larger. It is apparent that the Royco counter sensed the same magnitude of particles which were estimated to have been aerosolized, based on slurry count and the approximate 0.5 ml aerosolized.

It is readily apparent that as determined by the electronic particle counter, laminar-flow air circulation is remarkably efficient in clearing an environment of particulate contamination. For experiments 4, 5, and 6 the Royco counter which had been modified for rapid cleanup of particles within the system was unavailable and an unmodified Royco PC 200 was used. This may be responsible for some of the particles counted during these experiments following room clearance. There is also always the possibility that particles may be dislodged from surfaces of the room by the laminar airflow and that such particles may occasionally be monitored by the particle counters. This, however, is a condition which can easily be minimized by appropriate housekeeping procedures in ordinary use of such laminar-flow environments, a condition not possible under the experimental protocol used in these tests.

Table II shows the results of the effect of laminar airflow upon room clearance of viable particles as measured by Andersen samplers. Again it is evident that laminar-flow air circulation is exceedingly efficient in removing particulate contamination from the atmosphere. Only three viable particles in nine experiments were detected following room clearance for 1 minute, even though the samplers were functioned for 5 minutes following the room clearance. These results point up the efficiency of such systems in removing viable particulate contamination even more dramatically than do the results obtained from using the electronic particle counter.

One need not rely solely upon imagination to envision the utility of air conditioning systems with such efficiency in removing particulate contamination, both viable and non-viable. Such laminar-flow systems have already been widely utilized by the electronics industry for the assembly of minute components,⁵ by the pharmaceutical industry in the preparation and packaging of drug products,⁶ by the National Aeronautics and Space Authority for assembly of proposed interplanetary devices,⁷ and by the medical profession in at least one surgical room installation.⁸

* This counter functions at an air sampling rate of only 300 ml per minute; consequently, a multiplication factor of approximately 100 must be applied to convert the counts to cubic feet per minute (cfm).

These results confirm previous work and demonstrate with viable particles that properly operated laminar-flow air systems possess an almost absolute efficiency with respect to the rapid removal of such particles from the atmosphere. Such systems should be considered wherever it is desirable or necessary to reduce particulate contamination to an absolute minimum.

TABLE I

Royco Counts* Following Bacillus subtilis var. niger (globigii) Aerosolization
Before and After Room Clearance by Laminar-Flow Air Circulation

Experiment No.	1	2	3	4**	5**	6**	7	8	9
Slurry Dilution	1:10	1:10	1:5	1:100	1:100	1:100	1:1000	1:1000	1:1000
1st minute	4,879	6,967	11,050	696	268	298	59	---	34
2nd minute	4,420	7,203	10,552	754	294	319	41	---	24
3rd minute	4,130	7,117	10,874	773	293	285	39	---	23
4th minute	4,107	6,988	10,433	658	286	305	52	124	24
5th minute	4,103	6,548	10,143	648	279	274	43	122	29
Room circulation turned on for 1 minute before count of following samples									
1st minute	000	000	000	000	016	004	000	000	000
2nd minute	000	000	000	000	000	004	001	000	000
3rd minute	000	000	000	000	004	001	000	000	000
4th minute	001	000	000	000	000	001	000	016	000
5th minute	001	000	000	000	003	000	000	000	000
Estimated concentration of particles/ft ³ of room air	5.5×10^5	5.5×10^5	1.1×10^6	5.5×10^4	5.5×10^4	5.5×10^4	5.5×10^3	5.5×10^3	5.5×10^3

*Total particles 0.3μ and larger; 1-minute count; 300 ml/min sampling rate

** Royco Model PC 200 (unmodified)

TABLE II

Andersen Sampler Counts* Following *Bacillus subtilis* var. *niger* (globigii) Aerosolization
Before and After Room Clearance by Laminar-Flow Air Circulation

Experiment No.	1	2	3	4	5	6	7	8	9
Slurry Dilution	1:10	1:10	1:5	1:100	1:100	1:100	1:1000	1:1000	1:1000
Stage 1	91	174	420	15	9	12	2	0	1
Stage 2	132	208	426	21	27	24	3	1	1
Stage 3	364	333	>2427	27	38	56	1	2	1
Stage 4	>2427	>2427	>2427	172	214	289	25	11	9
Stage 5	>2427	>2427	>2427	>2427	>2427	>2427	931	1403	974
Stage 6	>2427	>2427	>2427	411	357	485	5	7	22
Total	>7868	>7996	>10554	3063	3072	3293	967	1424	1008
Room circulation turned on for 1 minute before count of following samples									
Stage 1	0	0	0	0	0	0	0	0	0
Stage 2	0	0	Broken	0	0	0	0	0	0
Stage 3	0	0	0	0	0	0	0	0	0
Stage 4	0	0	0	0	0	0	0	0	0
Stage 5	2	0	0	0	0	0	0	0	0
Stage 6	0	0	1	0	0	0	0	0	0
Total	2	0	1	0	0	0	0	0	0

* All colony counts corrected for "positive hole" value⁹

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